Salvianic acid A inhibits lipopolysaccharide-induced apoptosis through regulating glutathione peroxidase activity and malondialdehyde level in vascular endothelial cells

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[ABSTRACT] AIM: To find out the role of salvianic acid A (SAA) in the protection of vascular endothelial cells (VEC) and its possible mechanism in vitro. METHODS: The ingredient at various concentrations was added to human umbilical vein endothelial cells (HUVEC) treated with 0.5 µmol·L⁻¹ lipopolysaccharide (LPS) for 24 h. Apoptotic morphological changes of cells were observed under inverted phase contrast microscope; the cell viability was quantified using MTT assay. Nuclear fragmentation of cells was observed under laser scanning confocal microscope after being stained with acridinorange. Cell cycle distribution was detected by flow-cytometry after being stained with propidium iodide (PI). The activities of glutathione peroxidase (GPX-PX) as well as malondialdehyde (MDA) level in cells were measured by spectrophotometric methods as described in the assay kits. RESULTS: Apoptotic morphological changes and the decrease of cell viability of these cells were obviously inhibited by SAA in a dose-dependent manner. Furthermore, the abnormal cell cycle distribution, the decrease of GSH-Px activity and the increase of MDA level induced by LPS were markedly reversed. CONCLUSION: SAA exerts protective effect on VEC induced by LPS via an antioxidative mechanism.

[KEY WORDS] Salvianic acid A; Vascular endothelial cells; Glutathione peroxidase; Malondialdehyde; Lipopolysaccharide


1 Introduction

Salvia miltiorrhiza Bunge, which belongs to the Labiatae family, is a well-known Chinese traditional herbal medicine, has been widely used in oriental medicine for the treatment of cardiovascular diseases, such as atherosclerosis, hypertension and congestive heart failure, etc[1-2]. However, little is known about the underlying mechanism and the bioactive component of the above pharmacological effect of Salvia miltiorrhiza Bunge. According to several phytochemical reports, salvianic acid A, propionic acid, 3-(3, 4-dihydroxy-phenyl)-2- hydroxy (Fig. 1) is one main water-soluble component of Salvia miltiorrhiza. This component is capable of protecting some kinds of cells from apoptosis[3], but the role of SAA in the protection of vascular endothelial cells remains unknown.

Fig. 1  Structure of salvianic acid A

Lipopolysaccharide is a well-known VECs apoptosis inductor[4]. It induced apoptosis in VECs correlates with increasing oxidative stress[5]. Previous studies showed that many beneficial health properties of SAA are attributed to their antioxidant activities, and it is capable of protecting cells from damage caused by oxidative stress by inhibiting lipid peroxidation[3, 6]. In this study, we attempt to ravel the role of SAA in the protection of VECs treated by LPS, and find out its mechanism, focusing especially on regulating the abnormal oxidative stress induced by LPS.

2 Materials

2.1 Drugs

MCDB131 medium, heparin, fibroblast growth factor
cells 1 × 10^4 were seeded in each well of microtiter plate and baited with lipopolysaccharide 0.5 µmol·L\(^{-1}\) basal MCDB131 medium. (b) LPS group, cells were incubated with LPS 0.5 µmol·L\(^{-1}\), FGF and FBS respectively; SA*24, viability of cells treated with SAA 24 µmol·L\(^{-1}\). *P < 0.05, **P < 0.01 vs control, ##P < 0.01 vs LPS.

### 3.5 Activities of MDA, GSH-PX assay

The activities of glutathione peroxidase as well as maleic dialdehyde level in cells were measured by spectrophotometric methods as described in the assay kits.

### 3.6 Statistics analysis

Data are expressed as \( \bar{x} \pm s \). Significance testing was performed by means of Student’s \( t \)-test.

## 4 Results

### 4.1 Viability of VEC

24 h after the start of treatment, the viability of cells treated with lipopolysaccharide was 47.0 %. While in the presence of salvinic acid A, the viability of cells increased obviously, 64 %-73 %. The protective effect of 24 µmol·L\(^{-1}\) SAA was best (Fig. 2, \( P < 0.05 \)). Concentrations between 0.01 and 200 µmol·L\(^{-1}\) of salvinic acid A had no toxic effect on cell growth as observed by the MTT test (data not shown).

### 4.2 Apoptotic morphological changes in VEC

While the cells were incubated with LPS 0.5 µmol·L\(^{-1}\), specific morphological changes of apoptosis could be observed. Some cells gradually detached from the dish, some cells displayed wedge-like morphology on dish, and many apoptotic bodies were formed 24 h after the treatment. However, after exposure to salvinic acid A 20 µmol·L\(^{-1}\) for 24 h, the detachment and apoptosis in VEC were obviously inhibited, and few apoptotic bodies were observed (Fig. 3).

### 4.3 Nuclear fragmentation

As shown in Fig. 3, 24 h after the treatment with LPS, many nuclei became fragmented in the absence of SAA, but in the presence of this component the nuclear fragmentation were inhibited (Fig. 4).
Fig. 3 Morphological Micrographs (a) Cells cultured in the basal MCDB131 medium for 24 h; (b) Cells treated with LPS 0.5 µmol·L⁻¹ for 24 h, the detachment of cells and apoptotic bodies were noted; (c) Cells treated with both LPS 0.5 µmol·L⁻¹ and SAA 20 µmol·L⁻¹ for 24 h, the detachment of cells from dish was inhibited (X200)

Fig. 4 Effect of SAA on nuclear fragmentation. The cells were cultured under the same condition with Fig. 2 for 24 h, and were stained with acridinorange for 5 min, then were viewed using laser scanning confocal microscopy as described in the Materials and Methods. (A) nuclei of cells cultured in the basal MCDB131 medium; about 90% nuclei of cells maintain intact; (B) nuclei of cells treated with LPS 0.5 µmol·L⁻¹, the nuclear fragmentation of cells were noted; (C) nuclei of cells treated with both LPS 0.5 µmol·L⁻¹ and SAA 20 µmol·L⁻¹, the nuclear fragmentation of cells was markedly inhibited

4.4 The cell cycle distribution of VEC

Effects of salvianic acid A 20 µmol·L⁻¹ on the cell cycle of VEC were examined by flow-cytometry (Fig. 5). PI staining revealed that, in cells cultured in basic medium, the percentages of cells in S phase and G2/M phase were 15.34% and 3.85% respectively. After being treated with LPS 24 h, cell cycle distribution obviously changed, the S phase percentage decreased observably (2.76 %, P < 0.01), G2/M and

Fig. 5 Effect of SAA on the distribution of cell cycle. Flow cytometry profiles obtained from PI staining for determination of DNA content of dead or live cells, counteracting the cells with different content of DNA. (A) Analysis of DNA content in the VEC treated with three ways mentioned above, (a) VECs cultured in basic MCDB131 medium for 24 h. (b) and (c) VECs treated with LPS 0.5 µmol·L⁻¹ for 24 h with or without SAA 20 µmol·L⁻¹ respectively, (B) Table, The percentages of cells in various phases, G1 phase, S phase and G2/M phase. The result shown here is one representative experiment from two independent experiments. * P < 0.05, ** P < 0.01 vs control, † P < 0.05, ‡ P < 0.01 vs LPS
G1/G0 phase percentage both increased markedly (86.56% and 10.68%, \(P < 0.01\)). These data suggest that cells treated by LPS were arrested in G1/G0 and G2/M phase. Cells were not able to start DNA duplication and died during mitosis. When apoptosis of VEC was inhibited by SAA, the abnormal cell cycle distribution cells induced by LPS was evidently regulated. S phase distribution increased (13.98%, \(P < 0.01\)) and G2/M and G1/G0 phase distribution decreased (81.87% and 4.85%, \(P < 0.01\)) markedly. These results showed that SAA could regulate the abnormal cell cycle distribution of VECs induced by LPS.

4.5 Activity of GSH-PX and the concentration of MDA

Compared with the control group, the activity of GSH-PX in LPS group cells decreased evidently and the concentration of MDA was increased observably (\(P < 0.01\)). However, between SAA and the control group cells, differences in the activity of GSH-PX and the concentration of MDA are no apparent (Fig. 6A, B, \(P < 0.01\)).

![Image](https://example.com/image.png)

**Fig. 6** Effect of SAA on activities of glutathione peroxidase (GSH-PX, A) and maleic dialdehyde (MDA, B) Ctrl, the activity of GSH-PX or concentration of MDA in cells cultured in the basal medium; LPS, the activity of GSH-PX or concentration of MDA in cells treated with lipopolysaccharide 0.5 µmol·L⁻¹; SA 10 and SA 20, the activity of GSH-PX or concentration of MDA in cells treated with both LPS 0.5 µmol·L⁻¹ and salvianic acid A 10 and 20 µmol·L⁻¹ respectively. \(\bar{x} \pm s, n = 3, **P < 0.01 \ vs \ control, \ **P < 0.01 \ vs \ LPS\)

5 Discussion

It is well-known that VECs apoptosis has been implicated in the pathogenesis of many cardiovascular diseases[8], including atherosclerosis[9], hypertension[10] and congestive heart failure[11]. _Salvia miltorrhiza_ has been widely used in Chinese traditional medicine for treating such diseases for thousands of years[8]. However, the underlying mechanism and pharmacological active components of this herbal medicine remain unclear. It is reported that salvianic acid A has multiple biological activities including potent anti-inflammatory, antiatherosclerotic[9], so we tried to figure out the role of salvianic acid A in VECs protection. Our studies showed that, in vascular endothelial cells treated by lipopolysaccharide, apoptotic morphological changes, nuclear fragmentation and the decrease of cell viability were obviously inhibited by SAA. These results showed for the first time that SAA could obviously protect VECs from apoptosis induced by LPS. Protective effect of salvianic acid A on VECs partly accounts for the mechanism by which _Salvia miltorrhiza_ treats cardiovascular disease.

In response to stress or injury, obvious changes on the oxidative state could be detected in vascular endothelial cells[12]. An alteration in the balance of the pro- and antioxidant states, resulted in oxidative stress and apoptosis in VECs[12-14]. Studies showed that many beneficial health properties of SAA are attributed to their antioxidant activities, and it is capable of protecting cells from damage by inhibiting lipid peroxidation[6,3]. In the present study, we found that salvianic acid A could effectively reverse the decrease of GSH-Px activity and the increase of MDA level induced by LPS. These results demonstrate that SAA exerts protective effect onVEC injury induced by LPS via an antioxidative mechanism, and that the pharmacological potential of SAA against pathological processes related to oxidative stress. A variety of clinical pathological events have been found to be connected with oxidative stress injury. Hence antioxidant therapy has become an attractive therapeutic for this kind of diseases[15]. We suggest that SAA may be a bioactive and the potential new drug candidate against diseases mentioned above, so it is worthwhile to undertake further study in animal models and eventually in human prospective trials.

Apoptosis is closely linked to the regulation of cell cycle. Our previous studies demonstrated that, in vascular endothelial cells, when apoptosis are induced, cycle arrests predominantly at the G2/M and G1/G0 phase[17-18]. Our data are consistent with this notion, in VEC treated by LPS, the percentages of cells in S phase obviously decreased; the percentages of cells in G2/M and G1/G0 phase increased. When apoptosis was inhibited by SAA, the cell cycle distribution was significantly recovered. Results of the present study showed that SAA is able to regulate VEC apoptosis by affecting the cell cycle. It is proved that LPS mediates cell cycle progression and apoptosis by inducing the expression of the GADD45 genes family, which plays an important role in protecting cells against DNA damaged under various stress conditions, including oxidative stress[39]. The evidence leads us to find putative proteins that participate in apoptosis signaling mediated by SAA.

References


丹参酸 A 通过调控血管内皮细胞谷胱甘肽过氧化物酶活性及丙二醛含量抑制内毒素诱导的凋亡

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【摘 要】目的: 探寻丹参素(丹参酸 A, SAA)对脂多糖(LPS)处理的血管内皮细胞(VEC)的保护作用及可能机制。方法: 以 LPS 处理培养的人脐静脉血管内皮细胞, 造成细胞凋亡, 培养液中同时加入系列浓度的 SAA; 例置相差显微镜观察细胞形态变化; 同位素染色+激光共聚焦显微镜观测凋亡小体; PI 染色+流式细胞仪测定细胞周期分布; MTT 法检测细胞存活率; 生化试剂盒检测细胞内谷胱甘肽过氧化酶(GSH-Px)活性及丙二醛(MDA)水平。结果: SAA 可明显抑制细胞凋亡, 提高细胞存活率, 且该作用呈浓度依赖性。对于 LPS 导致的细胞周期异常、GSH-Px 活性下降、MDA 水平升高, SAA 均可明显逆转。结论: 丹参酸 A 通过抗氧化作用抑制内毒素诱导的血管内皮细胞凋亡。

【关键词】 丹参素; 血管内皮细胞; 内毒素; 谷胱甘肽过氧化物酶; 丙二醛